PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	B Part of It Protocol: A cluster randomised controlled trial to assess the impact of 4CMenB vaccine on pharyngeal carriage of Neisseria meningitidis in adolescents
AUTHORS	Marshall, Helen; McMillan, Mark; Koehler, Ann; Lawrence, Andrew; MacLennan, Jenny; Maiden, Martin; Ramsay, Mary; Ladhani, Shamez N.; Trotter, Caroline; Borrow, Ray; Finn, Adam; Sullivan, Thomas; Richmond, Peter; Kahler, Charlene; Whelan, Jane; Vadivelu, Kumaran

VERSION 1 – REVIEW

REVIEWER	Raquel Abad National Centre for Microbiology. Instituto de Salud Carlos III. Spain.
REVIEW RETURNED	02-Jan-2018

GENERAL COMMENTS	With the aim to assess the impact of 4CMenB vaccine on meningococcus nasopharyngeal carriage, an ongoing research study protocol is presented by the authors. The study design and methodology presented are appropriated and well defined. The dates of the study are included in the protocol; data collection will be complete throughout 2018. The study will provide relevant and useful information, since there are few data about this subject.

REVIEWER	Muhamed-Kheir TAHA Institut Pasteur, Paris, France
REVIEW RETURNED	02-Jan-2018

GENERAL COMMENTS	This is an important study that addresses the impact on carriage/acquisition of carriage of the 4CMenB vaccine (a vaccine against group B meningococci). The protocol is clear but some limitations need to be clarified and discussed. Specific comments: Introduction: 1-Page 7, first lines, the Authors may be willing to consider that the non-significant impact on carriage of MenB isolates may also be (if not mainly) due to low levels of expression of vaccine components in carriage isolates and/or low levels of bactericidal antibodies in nasopharynx. Methods: 2-As the Authors stated “a control group is essential”. The students are randomised per school (Fig.1). The two groups (vaccinated with 4CMenB) and the control (wait-listed group) are not independent with close and repeated contacts between the two groups. The

	expected significant impact of 4CMenB on carriage of genogroups BCWY from 3 months after dose 2, will result in lower circulation of any meningococci among all students impacting therefore on the control group. An independent control group may be needed as the primary objective is to compare overall carriage prevalence of disease causing genogroup between vaccinated and unvaccinated students. 3-The randomisation does not seem to consider the secretor status of ABO blood group antigens among participants (secretion versus non-secretion) nor this is scored in the questionnaire. The secretor status was suggested in several old works to impact on carriage (see Zorgani et al., FEMS Immunol Med Microbiol 14, 73 (Jun, 1996) and Blackwell et al., Epidemiol Infect 104, 203 (Apr, 1990). 4-The protocol will be based on PCR (using porA gene). If positive, sample will be cultured. This non-direct plating may reduce the yield of culture (see Roberts et al., J Infect 58, 103 (Feb, 2009). 5-porA-gene PCR may be negative due to the absence of porA gene (such isolates were also reported among disease isolates see van der Ende et al., J Infect Dis 187, 869 (Mar 1, 2003). 6-It is not clear what will be the results/methods that will be used for the final analysis to evaluate carriage (PCR, culture or both)? One may guess from the protocol that the Authors will use PCR. If so, how the results from the culture will be used? 7-PCR will not inform on the viability of the corresponding isolates. Detectable DNA by PCR may represent lysed (non-viable) but recently acquired meningococci. Discussion, 8- The MeNZB vaccine (an OMB-based vaccine) was introduced used in Australia between 2004 and 2008 to control the outbreak IMD due to B:P.P17-2,4 and 81% of individuals aged less than 20 years had received three doses of the MenB vaccine by April 2008 (see Arnold et al., Vaccine 29, 7100 (Sep 16, 2011). The 4CMenB contains the MeNZB. This point needs to be discussed and clarified in the protocol (randomisation, questionnaire, and analysis). 9-The discussion on the MATS data from disease isolates needs clarification as this may not be relevant to carriage isolates. No correlation is yet established between the level of antibodies needed for protection at the mucosal level and the levels of expression of the antigens targeted by the 4CMenB. Moreover, a recent work from Spain reported that quantifiable levels of two components of the 4CMenB (fHbp and NHBA) were found in only 10% and 75% of analyzed carriage strains.

REVIEWER	Robert Read University of Southampton
REVIEW RETURNED	02-Jan-2018

GENERAL COMMENTS	This is a very clear protocol description of a study which is similarl to the one published by myself and colleagues in 2014 (Read et al, Lancet). The difference is the large sample (about 12000 per arm) and the secondary school site (rather than university students) and a

	single 12 month post-vaccine oropharyngeal sample. The manuscript could be improved if the authors were able to provide an estimate of acquisition rate across the school years under study. As it stands they seem to be limited to data from a static cross sectional survey conducted in Queensland. If the authors have any data on the expected colonisation rate current in SA, and possible acquisition rates expected at their sites that would be very informative. Currently the reader has to assume that there will be sufficiently high rates of acquisition and displacement within these year groups to demonstrate an (/absence of) effect. I do not think that can be safely assumed. The Read study was conducted in a University environment with intense admixture of young people and a reasonable acquisition rate (except of serogroup B in that particular year!) which allowed an overall effect on carriage of approximately 20% to be observed. The authors might comment in the discussion on the single 12 month sampling point. One advantage of the Read study is that there were multiple sampling points over the follow up which provided considerable more data, albeit with a smaller sample.

REVIEWER	Matthew Snape University of Oxford, UK
REVIEW RETURNED	12-Jan-2018

GENERAL COMMENTS	Thank you for the opportunity to review this paper that outlines an important study. My specific comments are: - it is not clearly stated anywhere who is funding this study, and who are the sponsors - In the abstract, the term 'up to 24%' can include 0%, so I suggest this be re-worded. - Does 'wait-listed' mean delayed? - the introduction section 'carriage of N. meningitidis refers to nasopharyngeal carriage . Given meningococcal carriage studies generally take oropharyngeal swabs, perhaps this just be modified to just 'pharyngeal'? - page 7, line 2...I don't think that we can say there is a lack of evidence of effectiveness of a population programme given the UK data. - it would be helpful to the non-Australian reader to outline what age groups are in years 10, 11 and 12 in the Australian system (and that year 12 is the last year of secondary school - page 9, line 35....'Year 12 students are included as they are likely to have the highest carriage rates and to avoid any impact on any vaccine effect due to mixing of year levels'. I can see what you saying.....but it doesn't come across clearly. Perhaps.. ..., and mixing of unimmunised year 12 students with immunised year 10 and 11 students could potentially reduce any impact on MenB carriage. - Study processes....I had understood this study was also collecting saliva samples?

	- the phrase 'the three educational sectors' will not mean anything to those outside Australia - presumably SAEs will be reported to the study sponsor? - Under laboratory processes: the phrase 'Further molecular analysis will be used to determine the capsular group (A, B, C, W, X, Y).' is redundant is as this is gone into further detail later in the paragraph - 'Consistent with previous published carriage rates in school students,(25, 26) we estimate the carriage prevalence in unvaccinated South Australian adolescents will be 6-8 %'.....its not clear what is being referred to here (MenB, MenABCWXY). - it isn't clear how the demographic (risk factors) data collected at 12 months will be be brought into the analysis, and this would be an interesting issue to address - There is a heading of 'Laboratory Procedures' above a section relating to ethics review, which is likely to be an error. - 'This is a particularly important question for meningococcal vaccines due to the unique epidemiology of asymptomatic pharyngeal carriage and more critically important for protein-based MenB vaccines, where no such information exists'. This doesn't seem to take into account the Read et al study. - the authors will be aware of the UK MenB carriage study. While there are no publicly accessible references for this study as yet, this could be included under personal communication.

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author: Reviewer: 1 Reviewer Name: Raquel Abad Institution and Country: National Centre for Microbiology. Instituto de Salud Carlos III. Spain. Please state any competing interests or state ‘None declared’: None declared

With the aim to assess the impact of 4CMenB vaccine on meningococcus nasopharyngeal carriage, an ongoing research study protocol is presented by the authors. The study design and methodology presented are appropriated and well defined. The dates of the study are included in the protocol; data collection will be complete throughout 2018. The study will provide relevant and useful information, since there are few data about this subject.

Reviewer: 2 Reviewer Name: Muhamed-Kheir TAHA Institution and Country: Institut Pasteur, Paris, France Please state any competing interests or state ‘None declared’: None declared

This is an important study that addresses the impact on carriage/acquisition of carriage of the 4CMenB vaccine (a vaccine against group B meningococci). The protocol is clear but some limitations need to be clarified and discussed.

Specific comments: Introduction: 1-Page 7, first lines, the Authors may be willing to consider that the non-significant impact on carriage of MenB isolates may also be (if not mainly) due to low levels of

expression of vaccine components in carriage isolates and/or low levels of bactericidal antibodies in nasopharynx.

Response: We agree that there may be low levels of expression of vaccine components in carriage isolates and this is an important consideration in measuring 4CMenB vaccine impact on carriage. All cultured isolates will be whole genome sequenced and analysed for the presence of vaccine components. We have added the additional reason suggested by the reviewer for a non-significant finding in the Read et al study.

Methods: 2-As the Authors stated “a control group is essential”. The students are randomised per school (Fig.1). The two groups (vaccinated with 4CMenB) and the control (wait-listed group) are not independent with close and repeated contacts between the two groups. The expected significant impact of 4CMenB on carriage of genogroups BCWY from 3 months after dose 2, will result in lower circulation of any meningococci among all students impacting therefore on the control group. An independent control group may be needed as the primary objective is to compare overall carriage prevalence of disease causing genogroup between vaccinated and unvaccinated students.

Response: To clarify, schools are randomised, not students, therefore intervention and control groups are assumed to be independent. We assume the question addresses how much potential mixing there may be between vaccinated and unvaccinated students in different schools. All year 10,11,12 students in schools assigned to intervention will be offered the intervention. Whllst there may be mixing between vaccinated students in intervention schools and those in the same school who did not take part in the study, students in control schools are completely separate in location to intervention schools. In Australia there are limited opportunities for contact between intervention and control students outside competitive school sport. This would be over a very limited time of a few hours on a weekend day. It is possible that there may be small leakage where parents privately purchase 4CMenB for their children (control schools), but uptake in the private market is low in the adolescent age group. We have amended the figure to state “School randomisation” to better reflect the level of randomisation. We have added a new limitation to the protocol based on the reviewer’s suggestion.

3-The randomisation does not seem to consider the secretor status of ABO blood group antigens among participants (secretion versus non-secretion) nor this is scored in the questionnaire. The secretor status was suggested in several old works to impact on carriage (see Zorgani et al., FEMS Immunol Med Microbiol 14, 73 (Jun, 1996) and Blackwell et al., Epidemiol Infect 104, 203 (Apr, 1990).

Response: We do not have access to and students would be unaware of their secretor status of ABO blood group. As this is a RCT any impact on carriage in relation to secretor status would be expected to be equally distributed across both intervention and control groups. Additionally as this is a cluster RCT we are randomising according to cluster level characteristics not student characteristics.

4-The protocol will be based on PCR (using porA gene). If positive, sample will be cultured. This nondirect plating may reduce the yield of culture (see Roberts et al., J Infect 58, 103 (Feb, 2009).

Response: We agree this non-direct plating may reduce yield of culture by 10-20% but our primary outcome is PCR positivity not cultured isolates.

5-porA-gene PCR may be negative due to the absence of porA gene (such isolates were also reported among disease isolates see van der Ende et al., J Infect Dis 187, 869 (Mar 1, 2003).

Response: We expect to detect most carriage by detection of the porA gene. The majority of genogroups carried contain the porA gene. Whichever PCR target is used (e.g. fHBP, ctrA), there may be a small proportion of carriage that is not detected, however we anticipate this will be equal in

both groups due to randomisation, and believe porA is the most reliable target to detect N. meningitidis carriage. It is also the target used by the WHO reference group in carriage studies in Africa.

Response: We expect to detect most carriage by detection of the porA gene. The majority of genogroups carried contain the porA gene. Whichever PCR target is used (e.g. fHBP, ctrA), there may be a small proportion of carriage that is not detected, however we anticipate this will be equal in both groups due to randomisation, and believe porA is the most reliable target to detect N. meningitidis carriage. It is also the target used by the WHO reference group in carriage studies in Africa.

Response: The reviewer is correct PCR is the primary outcome as indicated on page 14. We have revised this sentence to better clarify this.

7-PCR will not inform on the viability of the corresponding isolates. Detectable DNA by PCR may represent lysed (non-viable) but recently acquired meningococci.

Response: This is possible but will not impact on our primary objective, which is to compare carriage prevalence as detected by PCR in vaccinated and unvaccinated students. As the study groups are randomised we do not expect there to be differences in the proportions of viable/non-viable isolates between the 2 groups. All isolates will undergo whole genome sequencing which will provide further information on impact of the 4CMenB on typable and non-typable carriage and on isolates containing vaccine antigens.

Discussion, 8- The MeNZB vaccine (an OMB-based vaccine) was introduced used in Australia between 2004 and 2008 to control the outbreak IMD due to B:P.P17-2,4 and 81% of individuals aged less than 20 years had received three doses of the MenB vaccine by April 2008 (see Arnold et al., Vaccine 29, 7100 (Sep 16, 2011). The 4CMenB contains the MeNZB. This point needs to be discussed and clarified in the protocol (randomisation, questionnaire, and analysis).

Response: The MenNZB vaccine was introduced in New Zealand, not in Australia, so this will not impact on our study.

9-The discussion on the MATS data from disease isolates needs clarification as this may not be relevant to carriage isolates. No correlation is yet established between the level of antibodies needed for protection at the mucosal level and the levels of expression of the antigens targeted by the 4CMenB. Moreover, a recent work from Spain reported that quantifiable levels of two components of the 4CMenB (fHbp and NHBA) were found in only 10% and 75% of analyzed carriage strains.

Response: Yes we agree MATS testing has been completed on disease causing isolates rather than carried N. meningitidis. We have removed the sentence to avoid any confusion.

Reviewer: 3 Reviewer Name: Robert Read Institution and Country: University of Southampton Please state any competing interests or state ‘None declared’: None Declared

This is a very clear protocol description of a study which is similar to the one published by myself and colleagues in 2014 (Read et al, Lancet). The difference is the large sample (about 12000 per arm) and the secondary school site (rather than university students) and a single 12 month post-vaccine oropharyngeal sample.

The manuscript could be improved if the authors were able to provide an estimate of acquisition rate across the school years under study. As it stands they seem to be limited to data from a static cross

sectional survey conducted in Queensland. If the authors have any data on the expected colonisation rate current in SA, and possible acquisition rates expected at their sites that would be very informative. Currently the reader has to assume that there will be sufficiently high rates of acquisition and displacement within these year groups to demonstrate an (/absence of) effect. I do not think that can be safely assumed. The Read study was conducted in a University environment with intense admixture of young people and a reasonable acquisition rate (except of serogroup B in that particular year!) which allowed an overall effect on carriage of approximately 20% to be observed.

The authors might comment in the discussion on the single 12 month sampling point. One advantage of the Read study is that there were multiple sampling points over the follow up which provided considerable more data, albeit with a smaller sample

Response; We thank reviewer 3 for their positive comments on the study and agree and very much appreciate the study design has been influenced by the findings of the published Read et al study. The studies are also different in the Read study used individual randomisation whereas this study randomises at the school level. We are very limited in carriage data in Australia, with the cited study in Queensland the only carriage study to pre-date our study. We are unable as yet to provide an estimate of acquisition of carriage in Australian school students. Jeppesen et al showed the acquisition rate of MenB carriage was 2.8 per 1000 person-months in their carriage study of senior school students. From our unpublished pilot study in 422 first year university students during their first few days on campus, carriage rates ranged from 6.2 to 8.2% 3 months later. We have not cited this study in the protocol paper as the paper on the pilot study results is still being drafted. We have not estimated an acquisition rate as the sample size is insufficient for us to do this. We agree acquisition may be lower in school students although data suggests strong intermixing, at senior school level with longer contact hours and opportunities for transmission. We have added the potential for low acquisition rates in this population to the limitations section.

We have added further discussion about the 12 month sample point as reviewer 3 suggests.

Reviewer: 4 Reviewer Name: Matthew Snape Institution and Country: University of Oxford, UK Please state any competing interests or state ‘None declared’: I am the Chief Investigator on a similar study that is about to commence in the UK. I am also on the scientific advisory board for the B part of it study.

Thank you for the opportunity to review this paper that outlines an important study.

My specific comments are:

- it is not clearly stated anywhere who is funding this study, and who are the sponsors

Response: Apologies for the omission – this has been added to the front page. Sponsor is The University of Adelaide, Funder is GlaxoSmithKline.

In the abstract, the term 'up to 24%' can include 0%, so I suggest this be re-worded.

Response: This has been reworded as suggested

- Does 'wait-listed' mean delayed?

Response: yes but we have revised to avoid any confusion

- the introduction section 'carriage of N. meningitidis refers to nasopharyngeal carriage. Given meningococcal carriage studies generally take oropharyngeal swabs, perhaps this just be modified to just 'pharyngeal'?

Response: thank you for pointing this out, we have revised the introduction as suggested

- page 7, line 2...I don't think that we can say there is a lack of evidence of effectiveness of a population programme given the UK data.

Response: This was in the context of the time of the application for funding when no population effectiveness data were available. It's important to explain reasons for the uncertainties raised by the PBAC – effectiveness and herd immunity. We have clarified this further in the paper
- it would be helpful to the non-Australian reader to outline what age groups are in years 10, 11 and 12 in the Australian system (and that year 12 is the last year of secondary school

Response; This has now been included in the methods section

- page 9, line 35....'Year 12 students are included as they are likely to have the highest carriage rates and to avoid any impact on any vaccine effect due to mixing of year levels'.

I can see what you saying.....but it doesn't come across clearly.

Perhaps..
..., and mixing of unimmunised year 12 students with immunised year 10 and 11 students could potentially reduce any impact on MenB carriage.

Response: Thanks for the suggestion we have revised as you suggested

- Study processes....I had understood this study was also collecting saliva samples?

Response: No, saliva samples were collected in the pilot university study not in this stud

the phrase 'the three educational sectors' will not mean anything to those outside Australia

Response: Thank you these are now defined in the protocol

- presumably SAEs will be reported to the study sponsor?

Response: SAEs are reported to the study sponsor and the vaccine manufacturer and the Therapeutic Goods Administration, Federal Government. We have clarified this in the protocol

- Under laboratory processes: the phrase 'Further molecular analysis will be used to determine the capsular group (A, B, C, W, X, Y).' is redundant is as this is gone into further detail later in the paragraph

Response: this sentence has been deleted

'Consistent with previous published carriage rates in school students,(25, 26) we estimate the carriage prevalence in unvaccinated South Australian adolescents will be 6-8 %'.....its not clear what is being referred to here (MenB, MenABCWXY).

Response: We are referring to overall carriage rates, as we don't have an estimate of individual genogroup carriage in Australia, we have clarified this in the protocol

- it isn't clear how the demographic (risk factors) data collected at 12 months will be brought into the analysis, and this would be an interesting issue to address

Response: The demographic and risk factor data will be analysed at 12 months to determine if there are any changes in risk factor profile.

- There is a heading of 'Laboratory Procedures' above a section relating to ethics review, which is likely to be an error.

Response: thank you this has been deleted

- 'This is a particularly important question for meningococcal vaccines due to the unique epidemiology of asymptomatic pharyngeal carriage and more critically important for protein-based MenB vaccines, where no such information exists'. This doesn't seem to take into account the Read et al study.

Response: We agree and have revised to "limited" rather than "no such' information exists

- the authors will be aware of the UK MenB carriage study. While there are no publicly accessible references for this study as yet, this could be included under personal communication.

Response: We are very happy to refer to the UK MenB carriage study and include in the discussion

VERSION 2 – REVIEW

REVIEWER	Matthew Snape University of Oxford, UK
REVIEW RETURNED	08-Mar-2018

GENERAL COMMENTS	All my comments have been adequately addressed.

REVIEWER	Muhamed-Kheir Taha Institut Pasteur, Paris, France
REVIEW RETURNED	08-Mar-2018

GENERAL COMMENTS	I thank the Authors for addressing satisfactorily the points raised in the first Reviewing round. I am sorry for the mixing between New Zealand and Australia for MenNZB vaccine One point may still need more optional clarification. 1-The use of porA PCR: It may be worthy to add another target such as sodC

VERSION 2 – AUTHOR RESPONSE

Reviewer Name: Muhamed-Kheir Taha

1-The use of porA PCR: It may be worthy to add another target such as sodC.

Response: Thank you for the suggestion to add another target such as SodC, which was considered during development of the study protocol. We decided on using a single target for the RCT, using PorA which was considered to be the most appropriate target due to its specificity for Neisseria meningitidis and also taking into consideration the size and complexity of the study and additional cost incurred using multiple targets. We were also concerned about the specificity of SodC, however we will consider testing a smaller proportion of the samples using a second target if another suitable target is identified. As this would be exploratory and dependent on funding we have not included this in the protocol.

Editorial changes have been made and are highlighted in yellow in the attached version 7. a tracked changes version has now been added. Please not abstract word count had to be reduced with addition of ethics and dissemination statement to abstract